201-14498

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2003 MAY 27 AM 10:19

May 23, 2003

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Subject: Comments on the HPV test plan for the metal carboxylates category

Dear Administrator Whitman,

The following are comments on the test plan for the metal carboxylates category, prepared by the Metal Carboxylates Coalition (the Coalition). This category is composed of 15 organic salts and 5 groups of closely related salts. These comments are submitted on behalf of People for the Ethical Treatment of Animals (PETA), the Physicians Committee for Responsible Medicine (PCRM), the Humane Society of the United States, the Doris Day Animal League, and Earth Island Institute. These animal, health, and environmental protection organizations have a combined membership of more than ten million Americans.

We note with concern that the EPA has, once again, posted its comments on this test plan *prior to* the close of the public comment period, thus ensuring that the agency does not take into account our concerns relating to animal welfare or the scientific issues we raise.

GENERAL COMMENTS

Our first concern is that the test plan, which proposes killing more than 1,000 animals in five or six tests, is of an unusually poor quality. It contains numerous spelling and grammatical errors, some of which obscure the meaning of the text. Other mistakes appear to be the result of simple carelessness, which concerns us greatly when animals lives are at stake. For example, with respect to ecotoxicity, the test plan states that acute aquatic toxicity studies will not be carried out, but a reproduction study will be carried out instead (p. 27). We do not know what this means, although we guess from the context that the intention might have been to refer to a chronic *Daphnia* study.

With respect to one test, the test plan is inconsistent as to whether it will actually be carried out: on page 23, the test plan states that an acute fish test will be carried out with cobalt neodecanoate, and the data obtained will be read across to the other members of subcategory 4. On the other hand, Table I (p. 29) states that this endpoint will be filled with existing data for dissociation products . The Coalition s test plan needs to be reviewed and revised carefully before proposing to kill any more animals.

In addition to the carelessness of its preparation and the poor quality of its English, the reasoning in this test plan is confused. Above all, the basic rationale for the metal carboxylates category is unclear. The aim of using categories is usually to reduce the amount of testing performed, since

categories enable data available for one or more members of the category to be read across to other members, so tests need not be carried out for the latter. However, the metal carboxylates category does not fulfill this function: it is divided into six subcategories, and data are not read across between subcategories. The test plan states, Testing needs for each subcategory are considered independently and are designed to allow each subcategory to stand alone (p. 16). Therefore, each subcategory is treated as a full category, and it is misleading to use the term category for metal carboxylates as a whole. Furthermore, even within each subcategory, the Coalition does not propose reading data across in a consistent manner. For example, within subcategory 2, reproductive and developmental toxicity data are available for five members, yet the Coalition does not propose reading these data across to the sixth member. Therefore, not even the subcategories are strict categories in the sense in which this term is generally used in the HPV program. The Coalition does not appear to have established consistent criteria for whether data may be read across between two given compounds.

We therefore urge the EPA to require that this test plan be rewritten and resubmitted. The term category should be restricted to groups of compounds amongst which at least some data can be read across, and the term subcategory should be restricted to groups among which all data can be read across between any two compounds.

The discussion below relates to the test plan as it stands, and, bearing in mind the test plan s many defects, this discussion must be considered tentative. The proposed animal tests, which are expected to kill 120-360 fish and at least 750 mammals, are as follows:

Salt	Fish test	Mammalian tests
Cobalt propionate (CAS no. 1560-69-6)	Acute test	-
Tin 2-ethylhexanoate (CAS no. 301-10-0)	Acute test	-
Zirconium 2-ethylhexanoate (CAS no. 22464-99-9)	-	Reproductive/developmental toxicity test
Cobalt neodecanoate (CAS no. 27253-31-2)	Acute test (uncertain; see above)	Acute oral toxicity test Genotoxicity test

First of all, it is clear that wider subcategories could be used. For example, we recommend that all salts in which the carboxylate moiety is aliphatic, saturated and unsubstituted should be included within a single category or subcategory. Among aliphatic, saturated, unsubstituted carboxylic acids, toxicity usually decreases with chain length, and exceptions to this rule are readily predictable on the basis of stereochemistry. Therefore, with respect to the carboxylate moiety at least, if toxicity data are available for acetates or propionates, similar data will not be needed for 2-ethylhexanoates or neodecanoates, which have much longer chains. Therefore, in the cases of the two longer-chain carboxylates for which fish tests are (or may be) proposed, tin 2-ethylhexanoate and cobalt neodecanoate, there should be an assessment of whether the necessary information is provided by the toxicity data for cobalt propionate.

In addition, the physicochemical properties of the salts should be determined before proposing to conduct any other tests. Factors such as hydrolysis, degree of ionization, water solubility, and

overall chemical behavior are likely to affect toxicity, and this possibility should be considered. The data that are already available should be summarized in the test plan, to obviate the need for the reader to search the 814-page-long summaries document for every item of information.

Finally, far more data are needed on the use of and human exposure to metal carboxylates. The annual numbers of people in the USA exposed to these compounds range from moderate (4,769) for tin 2-ethylhexanoate to extremely high (236,628) for aluminum tristearate (NIOSH). Because of the interspecies variability generally seen in toxicology, human data are more useful than animal data, yet the Coalition not only has no plans to carry out epidemiology studies, but it has disregarded the human exposure and epidemiology data that are already available. For example, cobalt acetate has been shown to give rise to hypertrophic effects in the upper respiratory tract, contact dermatitis, and cardiac disorders (Talakin 1990), and cobalt 2-ethylhexanoate and cobalt naphthenate can cause contact dermatitis (Bedello 1984, Foussereau 1988, Schena 1995, Kanerva 1996).

SPECIFIC COMMENTS

In addition to our general criticisms, we have the following specific criticisms of the proposed tests:

A. Fish tests on cobalt propionate and tin 2-ethylhexanoate (and possibly cobalt neodecanoate)

1. The octanol/water partition coefficients may be too high

The EPA states that fish tests are only appropriate if the log $K_{o/w}$ value of the test compound is less than 4.2 (EPA *Federal Register*, Dec. 26, 2000, p. 81695). The octanol/water partition coefficients of the metal carboxylates are not known, as determination of this parameter is proposed for all 20 test substances (test plan, pp. 18, 20-24, 27). It is premature to propose a fish test until these values have been determined.

2. In vitro and in silico methods are available

As in our comments on more than 30 previous test plans in the HPV program, we urge the Coalition to use alternatives to the acute fish toxicity test, such as ECOSAR, TETRATOX, or the recently validated *Dar*T test (see Appendix).

3. The ecologic relevance of fish toxicity should be taken into consideration

The purpose of fish tests is not for predicting toxicity in individual fish, but for predicting economic loss (to commercial and sport fisheries) and ecologic damage (fish are an important part of the food chain). The test therefore aims to show whether pollution with metal carboxylates will result in large-scale fish death. However, water pollution can wipe out fish stocks even with no direct toxicity, because killing the food of the fish will lead to starvation. Carps and catfishes are herbivorous, eating mostly algae, whereas most other familiar North American freshwater fish species are carnivorous, eating worms, small

crustaceans, smaller fish, insect larvae, etc. However, the toxicity of cobalt propionate, tin 2-ethylhexanoate and cobalt neodecanoate towards these types of organism is unknown, as shown by the inclusion in the test plan of tests of these salts on an aquatic crustacean (*Daphnia*) and an alga (pp. 19, 20, 23). Fish tests should not be carried out while other types of aquatic toxicity are unknown.

B. Reproductive/developmental toxicity test on zirconium 2-ethylhexanoate

The test proposed for zirconium 2-ethylhexanoates is on one of its dissociation products, zirconium (p. 21). However, animal data on reproductive and developmental toxicity are notoriously unreliable for predicting effects in humans, and use should therefore be made of two alternative approaches:

1. *In vitro tests*: A validated *in vitro* assay method is available (see Appendix).

2. *Epidemiology studies*: The annual exposure to zirconium 2-ethylhexanoate is over 60,000 people, including more than 4,500 women (NIOSH). This provides an appropriate opportunity for an epidemiology study.

C. Acute toxicity test on cobalt neodecanoate

In the test plan, although this is not explicitly stated, ecotoxicity generally refers to the toxicity of the salt, whereas human toxicity refers to the toxicity of its dissociation products. This is certainly a reasonable approach, as metal carboxylates almost completely dissociate at low pH values, such as that of the gastric juice, to which all orally administered toxins are rapidly exposed, whereas they show less dissociation under the higher-pH conditions obtaining in most environments (test plan, pp. 10-13). However, on this basis, it is difficult to understand why the following proposal is made: Acute toxicity, bacterial mutagenicity and a chromosome [*sic*] aberration data will be generated with neodecanoic acid, Co salt (p. 23). Additional data for the dissociation products are not required, because (i) acceptable reproductive and developmental data for the cobalt ion are already available (see summaries), as shown by the non-requirement for data for this ion for subcategories 1, 2, 3 and 6 (test plan, pp. 19, 21, 22, 28, respectively), and (ii) data for neodecanoate are currently being generated under the HPV program for the neo acids C5-C28 category. It therefore looks as though the data to be obtained for cobalt neodecanoate are for the salt rather than the dissociation products, but this is not clearly stated, and no explanation is given. This again epitomizes the poor reasoning to be found throughout this document. When the revised test plan is submitted, it should include the criteria for deciding whether data are required for the salt or for the dissociation products.

Although it is premature to discuss the need for acute toxicity data, we must point out that such data can be generated using an *in vitro* test, as detailed in the Appendix, and that the EPA has requested that the *in vitro* test be used prior to conducting an *in vivo* acute toxicity test.

D. Genotoxicity test on cobalt neodecanoate

As pointed out with respect to the proposed acute toxicity test, the justification for mammalian tests on cobalt neodecanoate is not clear, and any discussion of the proposed tests is therefore premature. In any case, the EPA has issued an official statement, both in the October 1999 animal welfare agreement and in the *Federal Register*, that *in vivo* genotoxicity tests should only be used if known chemical properties preclude the use of an *in vitro* test:

> Persons who conduct testing for chromosomal damage are encouraged to use *in vitro* genetic toxicity testing (Mammalian Chromosomal Aberration Test) to generate needed genetic toxicity screening data, unless known chemical properties preclude its use. These could include, for example, physical properties or chemical class characteristics. With regard to such cases, test sponsors are asked to submit to EPA the rationale for conducting one of these alternative tests as part of the test plan. (EPA *Federal Register* 2000, p. 81695)

Therefore, the Coalition should conduct an *in vitro* genetic toxicity test or provide the rationale for not doing so.

To conclude, we urge the EPA to require the Coalition to go back to the drawing board on this highly inadequate test plan and submit a plan that is complete, consistent, rational, and written in comprehensible English. We look forward to the opportunity to comment on a revised version of the test plan.

Thank you for your attention to these comments. We can be reached via e-mail at RichardT@PETA.org.

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Appendix: *In vitro* and *in silico* test methods

1. Fish acute toxicity tests

> TETRATOX, an assay based on the protozoan *Tetrahymena pyriformis* (Larsen 1997), is an appropriate method for use in this plan. With 50% growth impairment as the endpoint, the results of this assay show close similarity to toxicity in the fathead minnow (Schultz 1997). The extensive available information demonstrates that TETRATOX is an effective

alternative to fish testing. It is in fact already used extensively in industry, and is being considered for regulatory acceptance by the OECD. It is also rapid, easy to use, and inexpensive.

The recently validated *Dar*T test is another prospective replacement for *in vivo* tests. The test protocol and performance parameters are given in detail in Schulte (1994) and Nagel (1998). Briefly, however, the *Dar*T test uses fertilized zebrafish (*Danio rerio*) eggs as a surrogate for living fish. The exposure period is 48 hours, and endpoints assessed include coagulation, blastula development, gastrulation, termination of gastrulation, development of somites, movement, tail extension, eye development, circulation, heart rate, pigmentation and edema. Endpoints comparable to *in vivo* lethality include failure to complete gastrulation after 12 hours, absence of somites after 16 hours, absence of heartbeat after 48 hours, and coagulated eggs. The other endpoints provide further insight for a more detailed assessment of test substances. The reliability and relevance of the *Dar*T test have recently been confirmed in an international validation study coordinated and financed by the German Environmental Protection Agency; predictions of acute toxicity from the *Dar*T test were highly concordant with *in vivo* reference data (Schulte 1996). This *in vitro* test has been accepted in Germany as a replacement for the use of fish in the assessment of wastewater effluent (Friccius 1995), and is clearly suitable for immediate use as a replacement for the use of fish in the HPV program s screening-level toxicity studies.

With respect to *in silico* methods, several quantitative structure-activity relationship (QSAR) programs for estimating toxicity to fish and other aquatic organisms are available. The EPA itself encourages the use of one established QSAR: ECOSAR (See http://www.epa.gov/oppt/newchems/21ecosar.htm; EPA 2002a).

2. *Mammalian reproductive/developmental toxicity test*

An *in vitro* embryotoxicity test method, the rodent embryonic stem cell test, has recently been validated by the European Centre for the Validation of Alternative Methods, and the Centre s Scientific Advisory Committee has concluded that this test is ready to be considered for regulatory purposes (Genschow 2002). This test is now commercially available in the U.S. We therefore urge the Coalition to consider the use of this *in vitro* test. If a positive result is found in the embryonic stem cell test, zirconium 2-ethylhexanoate should be treated as a developmental toxicant/teratogen, and no further testing should then be carried out within the screening-level program. Although we have written to the EPA repeatedly concerning the inclusion of the embryonic stem cell test in the HPV Program, with correspondence dating back more than eight months, we have received no reply. We urge the Coalition to correspond directly with the EPA on the incorporation of this validated non-animal test.

3. *Mammalian acute toxicity test*

The most appropriate *in vitro* assay for mammalian acute toxicity is the basal cell cytotoxicity test. This involves assessing the effects of compounds on the viability of

human basal keratinocytes. This viability is determined from the intensity of staining by neutral red (a dye), which is taken up by healthy cells more than by dead and low-viability cells. Furthermore, in the Multicentre Evaluation of *In Vitro* Cytotoxicity (MEIC), a worldwide study organized by the Scandinavian Society for Cell Toxicology, basal cytotoxicity assays were found to be more reliable predictors of human lethal doses, for 50 reference chemicals, than were rodent LD_{50} values (Clemedson 1996a, 1996b, 1998a, 1998b, 2000, Ekwall 1998a, 1998b), and when certain other human toxicokinetic data, such as blood-brain barrier passage and timing of lethal action, were used in conjunction with the cytotoxicity results, the prediction of human lethal concentrations improved still further (Ekwall 2000). The EPA has released a statement encouraging companies participating in the HPV program to use the human keratinocyte cytotoxicity assay as a supplement to the *in vivo* acute toxicity assay, and, for setting initial doses, it has explicitly instructed that this *in vitro* test should be conducted prior to any *in vivo* acute toxicity testing (EPA 2002b).

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